

## REMARKS

Reconsideration and withdrawal of any rejections of the application, and allowance of the claims, especially in view of the remarks made herein are respectfully requested.

Claims 1-5 and 7-28 are pending in this application. Claims 1, 7, 11, 12 and 14 have been amended; claims 18-28 have been added; claims 6 and 17 have been cancelled.

It is submitted that the claims herewith and the claims as originally presented are and were in full compliance with the requirements of 35 U.S.C. §§101, 102, 103 and 112. The amendments to the claims herein are not made for the purpose of patentability within the meaning of 35 U.S.C. §§ 101, 102, 103 or 112; but rather the addition and amendments to the claims are made simply for clarification and to round out the scope of protection to which Applicants are entitled. Support for the new claims is found throughout the specification and from the originally-filed claims; no new matter is added.

## THE OBJECTIONS ARE OVERCOME

### **Drawings**

Figures 4 and 5 were objected to as allegedly being of insufficient quality for examination. Attached are formal drawings and a petition under 37 CFR 1.84 requesting the acceptance of color photographs. A proposed amendment to insert the required language is included herein. Reconsideration and withdrawal of the objection to the drawings are requested.

### **Specification**

The specification has been amended to incorporate the proper use of trademark notations and to correct spelling errors. The Examiner is thanked for his careful reading of the specification. Reconsideration and withdrawal of the objections to the specification are requested.

### **Claims**

Claims 1-13 and 17 were objected to because of various informalities. Claim 1 has been amended to recite only "PS" in parentheses. Claims 7, 11 and 12 have been amended to correct the punctuation. Claim 12 has been amended so that it no longer recites "unwanted proliferation of prostate cells prostate carcinoma". It is noted that there is no change in scope as a result of these amendments, and therefore there should be no estoppel. Reconsideration and withdrawal of the claim objections are requested.

**THE REJECTIONS UNDER 35 U.S.C. § 112, 1<sup>ST</sup> PARAGRAPH ARE OVERCOME**

Claims 1-13 and 17 were rejected to under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. It is respectfully submitted that the present application does enable one skilled in the art to make and use the claimed invention.

The present invention relates to methods for the integration of photodynamic therapy (PDT) and differentiation therapy (DT) in the inhibition and analysis of unwanted cellular proliferation. The inventive methods comprise inducing differentiation in a proliferating cell (DT) and providing photosensitizer to the cell followed by irradiation and photoactivation of the photosensitizer within the cell (PDT). Integration of PDT with DT enhances the inhibition of unwanted cellular proliferation.

The clinical use of photosensitizers is well known in the art. To date, several thousand patients have been treated with PDT for a variety of neoplasms. Randomized clinical trials of this modality using Photofrin® were initiated as early as 1987. These first randomized trials were sponsored by Quadra Logic Technologies, Inc. (now QLT Phototherapeutics, Vancouver, Canada) and American Cyanamid Co. (Pearl River, New York), and compared the efficacy of PDT with that of other forms of therapy for bladder, esophageal, and lung cancers. Within the past 5 years, significant progress has been made worldwide in obtaining regulatory approval for a variety of indications. Currently, PDT with the photosensitizer Photofrin® is approved in at least 10 countries. Approval for treatment with other photosensitizers has been requested in the United States, Canada, and Europe.

It is well known in the art that PDT is a binary therapy, having the advantage of inherent dual selectivity. Firstly, an increased concentration of photosensitizer accumulates in target tissues. (Photosensitizers preferentially accumulate within proliferating cells, such as those comprising neoplastic tissues). Secondly, the irradiation can be specifically delivered to the target tissue in a controlled volume. Photoactivation will be limited to only those irradiated areas that have accumulated sufficient amounts of photosensitizer. Thus, even if the photosensitizer does bind to normal tissue, the tissue will either not be targeted for irradiation or not contain the threshold level of photosensitizer necessary for photoactivation.

The Office Action admits that the application and Declaration by Dr. Ortel, filed August 27, 2002 in Paper No. 10, show that the invention can be practiced in both androgen-responsive prostate cancer cells and a mammary carcinoma. As stated in MPEP §2164.02, “[f]or a claimed

genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art... would expect the claimed genus could be used in that manner without undue experimentation." Applicants cannot be expected to exemplify and provide data for every type of condition that can be treated using the methods of the invention.

According to the Court of Appeals for the Federal Circuit in the case of *In re Wands*, 8 U.S.P.Q. 2d 1400 (Fed. Cir. 1988),

Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is undue, not experimentation.' The determination of what constitutes undue experimentation in a given case requires the application of standard of reasonableness, having due regard for the nature of the invention and the state of the art. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed ... [Citations omitted].

*Id.* at 1404.

Against this background, determining whether undue experimentation is required to practice a claimed invention turns on weighing many factors summarized in *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). For example, (1) the quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples of the invention; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims.

Undue experimentation is not required in this case. The Office Action asserts, on page 6, that "the declaration does not, nor does the specification provide a showing that the invention can be practiced to successfully treat a subject having an androgen-independent prostate cancer, which is not responsive to treatment with androgen." It is submitted that one of skill in the art would not try to treat an androgen-independent cancer with androgen.

It is further submitted that the claims do not require the administration of androgen; rather, claim 1 recites "inducing differentiation in a cell". The section of the application beginning on page 18, line 15, entitled "Differentiation Therapy" provides several examples of

agents that can be used to differentiate cells. From the teachings in the application and the knowledge in the art, the clinical use of differentiation factors is within the ability of the skilled artisan. As discussed in the specification, the retinoids have long been used in the clinical treatment of acute promyelocytic leukemia. See the specification, page 18, lines 20-24. Clinical use of differentiation therapy in the treatment of cancer and diabetes is well within the skill of one in the art from the teachings in the present application. See the specification, page 18, lines 20-31; page 19, lines 1-11. Thus, the differentiation factor selected can be any known in the art to cause differentiation of the proliferating cell type (e.g. retinoids for use with DT of hematopoietic disorders). It is well within the ability of the skilled artisan, using the teachings in the instant application, to recognize which agent will be effective, depending upon the nature of the unwanted proliferation, without undue experimentation.

The specification teaches methods of administering the differentiation factor (page 6, lines 8-12; page 8, lines 3-11) and the photosensitizer (page 8, lines 3-11; page 13, lines 12-19), including the timing and sequence of administration (page 6, lines 8-12; page 7, lines 8-17). The specification further teaches methods of photoactivation (page 13, lines 21-29). From the teachings in the application, coupled with the knowledge in the art, methods for administering photosensitizer compositions and carrying out photoactivation can be practiced without undue experimentation. See, for example, U.S. Patent Nos. 5,952,329, 5,807,881, 5,798,349, 5,776,966, 5,789,433, 5,736,563, and 5,484,803 (submitted as part of Paper No. 10). Clearly, as to Wands factor 2, there is ample guidance in the application; and, Wands factors 3, 5 and 6 are also in the Applicant's favor, as working examples are present, the state of the art is well-developed and the relative skill of those in the art is high.

The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *See In re Certain Limited-Charge Cell Culture Microcarriers*, 221 U.S.P.Q. 1165, 1174 (Int'l Trade Comm'n 1983), aff'd. sub. nom.; *Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104, 227 U.S.P.Q. 428 (Fed. Cir. 1985). In view of the foregoing, and the arguments and declaration of record, practicing the invention as claimed requires no undue experimentation.

Furthermore, in the rejection under Section 103, the Examiner's analysis of Mueller *et al.* and Santini *et al.* includes a discussion of differentiation agents used to treat breast cancer and leukemia. The conclusion, by the Examiner, is that “[o]ne of ordinary skill in the art at the time

the invention was made would have been motivated to use a differentiating agent that would cause the cells to differentiate in order to optimize the therapeutic effect.” It is submitted that the Examiner cannot have it both ways: the selection of a differentiation agent cannot be beyond the skill of the ordinary artisan and obvious at the same time. Clearly, the Office Action is admitting that Wands factors 7 and 8 are in the Applicants’ favor.

The Office Action states that claims 7 and 8 include a method further comprising administering a compound that causes the formation of a photosensitizer, and goes on to allege that “the specification does not teach the use of, or exemplify a compound that might be administered to the subject to cause the formation of a photosensitizer”. To the contrary, such compounds are disclosed in the paragraph beginning on page 13, line 12 of the application, e.g. porphyrin or porphyrin precursors. The specific example of ALA-induced proto porphyrin IX is disclosed on page 13, and is exemplified in Examples 1-6. Again, this demonstrates that Wands factors 2 and 3 are in Applicants’ favor.

The Office Action goes on to state that administration of a photosensitizer coupled to a targeting moiety, as claimed in claim 9, is not exemplified. As stated in the first line of MPEP §2164.02, “[c]ompliance with the enablement requirement of 35 U.S.C. 112, first paragraph, does not turn on whether an example is disclosed.” *In re Goffe*, 191 U.S.P.Q. 429, 431 (“To demand that the first to disclose shall limit his claims to what he has found will work or to materials which meet the guidelines specified for ‘preferred’ materials in a process . . . would not serve the constitutional purpose of promoting progress in the useful arts.”). Moreover, the specification contains an entire section regarding targeting moieties (page 14), and lists preferred targeting moieties, such as cell surface receptor ligands and antibodies.

Further, the method of claim 9 is not, as stated in the Office Action, analogous to cancer vaccination. The Office Action goes on to allege on page 7 that the efficacy of the approach “depends upon the effectiveness of tumor antigen-specific antibodies to ameliorate or inhibit tumors”. For the purposes of this invention, the targeting moiety is not used to ameliorate or inhibit tumors. Rather, it is used to target the PS to the cells undergoing unwanted proliferation. It is the PS that affects the proliferating cells, not the targeting moiety. The instant invention does not involve “antibodies to ameliorate or inhibit tumors”. Therefore, the rejections based upon the assumption that claim 9 deals with antibody therapy are misguided.

Claim 11 was rejected as allegedly lacking adequate written description and enablement.

Claim 11 was criticized as not being exemplified; as discussed above, enablement is not precluded by the lack of a working example. However, claim 11 has been amended to include the recitation that the antidiabetic compound or ligand for a transcription factor induces differentiation. The increased sensitivity to photodynamic therapy is due to differentiation, as demonstrated in Examples 2 and 3, and it is well within the abilities of the skilled artisan to select a compound that will induce differentiation from the teachings in the application and the knowledge in the art.

Claim 12 was rejected as allegedly lacking enablement and adequate written description. The Examiner objected to the recitation of unspecified hormonal and retinoic acid increasing agents. However, claim 12 has been amended to specify dihydrotestosterone or liarozole.

The Office Action further alleges that the teachings of the specification are not commensurate the scope of the claims because the art of drug discovery is unpredictable. MPEP 2164.02(c) provides guidelines to follow in determining whether a showing of success by an applicant using *in vitro* data supports claims directed to the analogous *in vivo* application. An *in vitro* model is acceptable where it is recognized in the art that this model correlates to a specific *in vivo* condition. If this is has not yet been established in the art, the *in vitro* model is acceptable if one skilled in the art would accept the model as *reasonably* correlating to the condition. The “reasonableness” standard serves to prevent the PTO from unnecessarily and inappropriately adopting the more stringent standards of the FDA.<sup>1</sup>

In the present invention, the “condition” of MPEP 2164.02(c) is any condition characterized by unwanted cellular proliferation in a subject. The “condition” can be alleviated by inhibition of the unwanted cellular proliferation. When testing a therapy for inhibition of unwanted cellular proliferation, a good experimental model should test whether 1) the therapy will target the cells of interest and whether 2) the proliferation is inhibited as a result. If this test is carried out in *in vitro* and it is successful, the result is reasonably predictive of success *in vivo*. For the inventive methods, results *in vitro* have been shown to correlate with results *in vivo*.

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<sup>1</sup> Public hearings were held in San Diego on October 17, 1994, where then PTO Commissioner Bruce Lehman and other PTO representatives received comments on the inappropriate standards that Examiners were applying to biotechnological inventions and as a result of these and other objections raised by the scientific community, the present “reasonableness” standard is now applied.

All heme precursors (beginning with ALA) can be converted into protoporphyrin IX.

The conversion of protoporphyrin IX is catalyzed by ferrochelatase. Therefore, catalysis of protoporphyrin IX will be uniform where ferrochelatase is present, regardless of the setting (i.e., in vitro settings are analogous to in vivo settings where ferrochelatase is present). The conversion is predictable. As much was shown in the declaration of Dr. Ortel, filed on August 27, 2001.

The Declaration of Dr. Ortel and the teaching in the specification show that DT/PDT will successfully inhibit unwanted proliferation in neoplastic tissues *in vitro* and *in vivo*. Further, DT/PDT can be carried out with specificity as well as potency. As discussed above and in the Declaration, there are at least three levels of selection contributing to the specificity of the inventive methods:

- The differentiation factor will selectively target proliferating cells.
- The photosensitizer will selectively target proliferating cells and as a result, the proliferating cells accumulate substantially greater amounts of photosensitizer.
- The activating light can be targeted directly to the proliferating cells, and delivered in amounts specific to the level of photosensitizer that has accumulated only in those cells.

Thus, the inventive methods can be practiced with specificity and potency by one of skill in the art and without the need for undue experimentation.

The Applicants should not be required to provide further experimental data. Given the strong showing of success both *in vitro* and *in vivo* with the inventive methods, and the positive correlation between *in vitro* and *in vivo* results, the full scope of the claims, as amended, is allowable. A rigorous or an invariable exact correlation is not required, as stated in *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 U.S.P.Q. 739, 747 (Fed. Cir. 1985) ("based upon the relevant evidence as a whole, there is a reasonable correlation between the disclosed in vitro utility and an in vivo activity, and therefore a rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence); *see also In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 1441 (Fed. Cir. 1995) (reversing the PTO decision

based on finding that *in vitro* data did not support *in vivo* applications). Further experimentation, such as in human subjects, is clearly not required. The PTO is not the FDA.

Claims 14-16 were rejected because a cell having unwanted proliferation is allegedly unclear. The method of claim 14 now recites “detecting the presence of a disorder characterized by unwanted cell proliferation”. Further, the difference detected between the cell having unwanted proliferation and a control cell is specified, namely, an increase in light emission. Support for this amendment can be found on page 19, lines 14-15.

Also, the Examiner appears to dispute, on page 12 of the Office Action, that the amount of protoporphyrin that accumulates in a cell having unwanted proliferation differs from that in a control cell. As explained by Cabib *et al.* (U.S. Patent No. 5,784,162, cited by the Examiner), the photosensitizer protoporphyrin (PP) is “highly selective” for abnormally dividing cells, such as tumor cells, due to the “markedly elevate PP biosynthesis and accumulation in the fast-dividing transformed-cells in comparison to the surrounding normal tissue.” (See column 46.) Therefore, the assertion that the differentiation agent would have to have disparate effects upon the cell having unwanted proliferation and the control cell is misguided. The difference in sensitivity of the two types of cells to photodynamic therapy already exists and the addition of a differentiating agent simply intensifies this difference.

Reconsideration and withdraw of the rejections under the first paragraph of 35 U.S.C. §112 are respectfully requested.

#### **THE REJECTIONS UNDER 35 U.S.C. § 112, 2<sup>ND</sup> PARAGRAPH ARE OVERCOME**

Claims 1-17 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. The Office Action states that the term “unwanted”, recited in claims 1, 2, 12 and 14 is subjective. The rejection on this basis is traversed. The specification clearly defines and gives examples of disorders characterized by unwanted cell proliferation. See, for example, the paragraph beginning on page 8, line 25, the paragraph beginning on page 10, line 6, and the paragraph beginning on page 19, line 30. While the Examiner contends that “what may be considered ‘unwanted’ by one person, may not be considered so by another”, it is respectfully submitted that the disorders listed in the above-cited paragraphs would be unwanted by anyone.

Claim 12 was rejected for reciting “which increases levels of retinoic acid”. Claim 12 has been amended to eliminate this recitation.

Claims 14-16 were rejected for reciting “characterized as having unwanted proliferation”. Claim 14 has been amended to recite a “disorder characterized by unwanted cell proliferation”, which, as discussed above, is clearly defined at several points in the specification.

Claims 14-16 were also rejected for reciting “detecting a difference between the cell and a control cell”. Claim 14 has been amended to clarify the difference being detected.

Claims 14-16 were further rejected for not reciting a positive process step that relates back to the preamble. Claim 14 has been amended to correct this.

Reconsideration and withdrawal of the rejections under §112, second paragraph, are requested.

### **THE REJECTIONS UNDER 35 U.S.C. § 102 ARE OVERCOME**

Claims 1-3, 6, 7 and 13 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Koulu. A 35 U.S.C. § 102 rejection must show that each and every element of the claimed invention is found in a single reference. The rejection is traversed, as Koulu does not disclose every element of these claims. Claim 1 recites a method that results in treating unwanted cell proliferation. Although Koulu used patients with psoriasis, the method was performed on “uninvolved skin”, not on psoriatic skin. The use of retinoid therapy was hypothesized to decrease the carcinogenic effects of radiation with ultraviolet light, and the author concluded that the treatment was not effective in doing so. “As shown in the Table, a simultaneous retinoid treatment neither augmented nor counteracted the LC-depleting effects of the PUVA exposures.” (p. 43) Therefore, the method of Koulu does not treat unwanted cell proliferation, as required by the language of claims 1-3, 6, 7 and 13.

Claims 1, 2 and 13-15 were rejected under 35 U.S.C. §102(e) as allegedly being anticipated by U.S. Patent No. 5,784,162 (“the ‘162 patent”). The ‘162 patent does not teach every element of claims 1, 2 and 13-15. The first step of independent claims 1 and 14 require the use of a differentiation agent. The Examiner maintains that dimethylsulfoxide (DMSO) is a “known differentiating agent”, however, he does not present any evidence of this assertion. DMSO is used by the inventors of the ‘162 patent, as described in Example 3, to chemically induce the photosensitizer; any effect on cellular differentiation are not taught. Example 8 does not teach the use of a differentiation agent either, but rather “illustrates a method for detecting an abnormal cell comprising providing a light-emitting agent to the cell, activating the agent, and detecting a difference between the cell and a control cell.” (Office Action, page 8)

Therefore, the cited documents do not teach every element of the claimed invention, and reconsideration and withdrawal of the Section 102 rejections are requested.

**THE REJECTION UNDER 35 U.S.C. § 103 IS OVERCOME**

Claims 1-4, 6-8 and 10-15 were rejected under 35 U.S.C. § 103, as allegedly being unpatentable over Ortel *et al.* and Momma *et al.* in view of Mueller *et al.* and Santini *et al.* It is submitted that Ortel *et al.* is not a prior art document. The attached Declaration states that Ortel *et al.* is not the work of others as defined by 35 U.S.C. §102(a). It is also not prior art under 35 U.S.C. §102(b); the priority date of this application is June 3, 1999, and the publication date of Ortel *et al.* is June 10, 1998, as reflected by the library's date stamp on the face of the journal (see attached). Therefore, Ortel *et al.* cannot be properly cited as prior art against the present application. (*See in re Katz*, 687 F.2d 450, 215 USPQ 14 (CCPA 1982)).

Reconsideration and withdraw of the rejections under 35 U.S.C. § 103 are respectfully requested.

**REQUEST FOR INTERVIEW**

If any issue remains as an impediment to allowance, an interview, with supervisory review, is respectfully requested prior to issuance of any paper other than a Notice of Allowance. The Examiner is additionally respectfully requested to telephonically contact the undersigned to arrange a mutually convenient time and manner for the interview. The Examiner is also invited to telephonically contact the undersigned if there are any minor, formal issues that need resolving prior to issuance of a Notice of Allowance, with a view towards resolving such minor, formal issues via telephonic interview.

**CONCLUSION**

Moreover, in view of the amendment, remarks, attachments and Declaration herewith, the application is in condition for allowance. Favorable reconsideration of the application and prompt issuance of a Notice of Allowance are earnestly solicited.

Respectfully submitted,

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification**

On page 2, line 29:

In preferred embodiments: the photosensitizer has a chemical structure that includes multiple conjugated rings that allow for light absorption and photoactivation, e.g., the photosensitizer can produce singlet oxygen upon absorption of electromagnetic irradiation at the proper energy level and wavelength.[:] [t]The photosensitizer can include a porphyrin, porphyrin derivative or analog thereof, e.g., a tetraphyrroles; or the photosensitizer can include chlorin e6, a chlorin derivative or analog thereof. Suitable photosensitizers include [p]Photofrin<sup>TM</sup>[®]; synthetic diporphyrins and dichlorins; hydrophorphyrins, e.g., chlorins and bacteriochlorins of the tetra(hydroxyphenyl) porphyrin series; phthalocyanines; O-substituted tetraphenyl porphyrins (picket fence porphyrins); 3,1-meso tetrakis (o-propionamido phenyl) porphyrin; Verdins; Purpurins, e.g., tin and zinc derivatives of octaethylpurpurin (NT2), and etiopurpurin (ET2); Chlorins, e.g., chlorin e6 and mono-1-aspartyl derivative of chlorin e6; Benzoporphyrin derivatives (BPD), e.g., benzoporphyrin monoacid derivatives, tetracyanoethylene adducts of benzoporphyrin, dimethyl acetylenedicarboxylate adducts of benzoporphyrin, Diels-Alder adducts, and monoacid ring “a” derivative of benzoporphyrin; Low density lipoprotein mediated localization parameters similar to those observed with hematoporphyrin derivative (HPD); sulfonated aluminum phthalocyanine (Pc) sulfonated AlPc disulfonated (AlPcS2) tetrasulfonated derivative sulfonated aluminum naphthalocyanines chloroaluminum sulfonated phthalocyanine (CASP); zinc naphthalocyanines; anthracenediones; anthrapyrazoles; aminoanthraquinone; phenoxyazine dyes; phenothiazine derivatives; chalcogenapyrylium dyes cationic selena and tellurapyrylium derivatives; ring-substituted cationic PC; pheophorbide; hematoporphyrin (HP); protoporphyrin; ALA; and ALA esters, hexyl ester or methyl ester.

On page 4, line 3:

In a preferred embodiment, the subject has an unwanted proliferation of prostate cells, e.g., prostate carcinoma. Preferred treatment includes administering PS and a hormonal agent, e.g., a retinoid or vitamin D, or an agent which increases levels of retinoic acid, e.g., liarozole or Liazal<sup>TM</sup>, to the subject.

On page 5, line 10:

In another embodiment, the light emitting agent is a PS. In preferred embodiments: the photosensitizer has a chemical structure that includes multiple conjugated rings that allow for light absorption and photoactivation, e.g., the photosensitizer can produce singlet oxygen upon absorption of electromagnetic irradiation at the proper energy level and wavelength. [;][t]The photosensitizer can include a porphyrin, porphyrin derivative or analog thereof, e.g., a tetraphyrroles; or the photosensitizer can include chlorin e6, a chlorin derivative or analog thereof. Suitable photosensitizers include [p]Photofrin<sup>TM</sup>[®]; synthetic diporphyrins and dichlorins; hydrophorphyrins, e.g., chlorins and bacteriochlorins of the tetra(hydroxyphenyl) porphyrin series; phthalocyanines; O-substituted tetraphenyl porphyrins (picket fence porphyrins); 3,1-meso tetrakis (o-propionamido phenyl) porphyrin; Verdins; Purpurins, e.g., tin and zinc derivatives of octaethylpurpurin (NT2), and etiopurpurin (ET2); Chlorins, e.g., chlorin e6 and mono-1-aspartyl derivative of chlorin e6; Benzoporphyrin derivatives (BPD), e.g., benzoporphyrin monoacid derivatives, tetracyanoethylene adducts of benzoporphyrin, dimethyl acetylenedicarboxylate adducts of benzoporphyrin, Diels-Alder adducts, and monoacid ring "a" derivative of benzoporphyrin; Low density lipoprotein mediated localization parameters similar to those observed with hematoporphyrin derivative (HPD); sulfonated aluminum phthalocyanine (Pc) sulfonated AlPc disulfonated (AlPcS2) tetrasulfonated derivative sulfonated aluminum naphthalocyanines chloroaluminum sulfonated phthalocyanine (CASP); zinc naphthalocyanines; anthracenediones; anthrapyrazoles; aminoanthraquinone; phenoxyazine dyes; phenothiazine derivatives; chalcogenapyrylium dyes cationic selena and tellurapyrylium derivatives; ring-substituted cationic PC; pheophorbide; hematoporphyrin (HP); protoporphyrin; ALA; and ALA esters, hexyl ester or methyl ester.

On page 7, line 4:

The differentiating agent can be, e.g., a hormonal agent, e.g. a retinoid or vitamin D, or an agent which increases levels of retinoic acid, e.g., liarozole or LiaZal<sup>TM</sup> retinoic acid; or an antidiabetic compound, e.g., troglitazone[troglituzone], or a ligand for a transcription factor, e.g., transcription factor PPAR gamma.

On page 11, line 29:

Photosensitizers include, but are not limited to, hematoporphyrins, such as hematoporphyrin HCl and hematoporphyrin esters (Dobson, J. and M. Wilson, *Archs. Oral Biol.* 37:883-887); dihematoporphyrin ester (Wilson, M. et al., 1993, *Oral Microbiol. Immunol.* 8:182-187); hematoporphyrin IX (Russell et al., 1991, *Can J. App. Spectros.* 36:103-107, available from Porphyrin Products, Logan , UT) and its derivatives; 3,1-meso tetrakis (*o*-propionamideophenyl) porphyrin; hydroporphyrins such a chlorin, herein, and bacteriochlorin of the tetra (hydroxyphenyl) porphyrin series, and synthetic diporphyrins and dichlorins; *o*-substituted tetraphenyl porphyrins (picket fence porphyrins); chlorin e6 monoethylendiamine monamide (CMA Goff, B.A. et al., 1994, 70:474-480, available from Porphyrin Products, Logan, UT); mono-1-aspartyl derivative of chlorin e6, and mono- and di-aspartyl derivative of chlorin e6; the hematoporphyrin mixture Photofrin™ II ([Quardra]Quadra Logic Technologies, Inc., Vancouver, BC, Canada); benzophorphyrin derivatives (BPD), including benzoporphyrin monoacid Ring A (BDP-MA), tetracyanoethylene adducts, dimethyl acetylene dicarboxylate. adducts, Diels-Alder adducts, and monoacid ring "a" derivatives; a naphthalocyanine (Biolo, R., 1994, *Photochem. and Photobiol.* 59:362-365); a Zn(II)-phthalocyanine (Shopora, M. et al., 1995, *Lasers in Medical Science* 10:43-46); toluidine blue O (Wilson, M. et al., 1993, *Lasers in Medical Sci.* 8:69-73); aluminum sulfonated and disulfonated phthalocyanine *ibid.*; and phthalocyanines without metal substituents, and with varying other substituents; a tetrasulfated derivative; sulfonated aluminum naphthalocyanines; methylene blue (*ibid.*); nile blue; crystal violet; azure  $\beta$  chloride and rose bengal (Wilson, M., 1994, *Intl. Dent. J.* 44:187-189). Numerous photosensitizer entitites are disclosed in Wilson, M. et al., 1992, *Curr. Micro.* 25:77-81, and in Okamoto, H. et al., 1992, *Lasers in Surg. Med.* 12:450-485.

On page 17, line 12:

The production and purification of light emitting agent:targeting moiety conjugates can be practiced by methods known in the art. Yield from coupling reactions can be assessed by spectroscopy of product eluting from a chromatographic fractionation in the final step of purification. The presence of uncoupled photosensitizer and reaction products containing the photosensitizer can be followed by the physical property that the photosensitizer moiety absorbs light at a characteristic wavelength and extinction coefficient, so incorporation into products can be monitored by absorbance at that wavelength or a similar wavelength. Coupling of one or

more photosensitizer molecules to a targeting moiety or to a backbone shifts the peak of absorbance in the elution profile in fractions eluted using sizing gel chromatography, e.g., with the appropriate choice of Sephadex™ G50, G100, or G200 or other such matrices (Pharmacia-Biotech, Piscataway, NJ). Choice of appropriate sizing gel, for example Sephadex™ gel, can be determined by that gel in which the photosensitizer elutes in a fraction beyond the excluded volume of material too large to interact with the bead, i.e., the uncoupled starting photosensitizer composition interacts to some extent with the fractionation bead and is concomitantly retarded to some extent. The correct useful gel can be predicted from the molecular weight of the uncoupled photosensitizer. The successful reaction products of photosensitizer compositions coupled to additional moieties generally have characteristic higher molecular weights, causing them to interact with the chromatographic bead to a lesser extent, and thus appear in fractions eluting earlier than fractions containing the uncoupled photosensitizer substrate. Unreacted photosensitizer substrate generally appears in fractions characteristic of the starting material, and the yield from each reaction can thus be assessed both from the size of the peak of larger molecular weight material, and the decrease in the peak of characteristic starting material. The area under the peak of the product fractions is converted to the size of the yield using the molar extinction coefficient.

On page 18, line 16:

Neoplasia involves the loss of normal regulatory mechanisms often associated with the differentiated state. Among a host of strategies to deal with neoplasia, differentiation therapy takes advantage of the fact that normal regulation can sometimes be restored by inducing terminal differentiation of the cancer cells (*Carducci et al., Seminars in Oncology*. 23:56-62, 1996). The best-known example of differentiation therapy is probably the use of retinoids in acute promyelocytic leukemia. For that particular malignancy, administration of retinoic acid induces immature promyelocytic cells to differentiate along the pathway toward more mature neutrophils, producing cells that are less proliferative and more responsive to adjunctive chemotherapy (Degos *et al.*, *Blood* 85:2643-53, 1995). The newest example of differentiation therapy may be the use of antidiabetic drugs (troglitazone), ligands for the nuclear transcription factor PPAR $\gamma$ , to stimulate terminal differentiation of malignant breast epithelial cells (Elstner *et al.*, *Proc Natl Acad Sci USA* 95:8806-11, 1998 and Mueller *et al.* *Mol Cell* 1:465-70, 1998). For prostate carcinoma, the notion of differentiation therapy has stemmed from observations that hormonal agents, principally retinoids and vitamin D, can induce differentiation markers in cell

lines derived from prostate tumors, e.g., the LNCaP line. Clinically, an agent (liarozole, Liazal™) which promotes cell differentiation by increasing intratumoral levels of retinoic acid is now in early clinical trials, and appears to have some promise for the treatment of prostate cancer. Examples of other differentiation agents include, but are not limited to, polar/apolar compounds such as hexamethylene bisacetamide; vitamin D analogs including 1,25-(OH)<sub>2</sub>[sub.2] D<sub>3</sub>[sub.3]; histone hyperacetylators such as sodium butyrate and prodrugs thereof; sodium propionate and trichostatin A; hormones such as glucocorticoids; antioxidants such as PDTC; peroxisome proliferators such as clofibrate; and miscellaneous differentiating agents such as phenylacetate and phenylbutyrate.

On page 21, line 25:

SCID mice bearing (orthotopic) LNCaP tumors were pretreated with a high dose of 400 µg/kg R1881 and after 4 days received 250 mg/kg ALA i.p. At 4 hours, the mice were sacrificed and the tumors removed. Frozen section of the tumors were imaged on the confocal laser scanning microscope (CLSM). Using 633 nm excitation, the sections of tumors from an untreated mouse (Figure 5A) and an R1881-pretreated mouse (Figure 5B) were imaged at the same instrument parameters.

### In the Claims

1. (Amended) A method of controlling[treating a subject having] unwanted cell proliferation in a subject in need thereof comprising:
  - inducing differentiation in a cell;
  - providing said cell with a photosensitizer ([a ]PS); and
  - activating said PS, whereby the cell is of the type of the cell proliferation to be controlled, thereby killing the cell and controlling[treating] the unwanted cell proliferation.
7. (Twice Amended) The method of claim 1, further comprising administration to the subject of a compound which causes the accumulation of a PS, the formation of a PS, or is converted to a PS[,] in the subject's body.
11. (Amended) The method of claim 1, wherein the subject has a malignancy of breast epithelial cells, and a PS and an antidiabetic compound[,] or a ligand for a transcription factor is administered to the subject, wherein the antidiabetic compound or ligand induces differentiation of the cells.

12. (Amended) The method of claim 1, wherein the subject has prostate carcinoma and wherein the[an] unwanted cell proliferation is of prostate cells [prostate carcinoma], and a PS and dihydrotestosterone or liarozole [a hormonal agent, or an agent which increases levels of retinoic acid] are administered to the subject.

14. (Amended) A method of detecting the presence of a disorder[a cell] characterized by[as having] unwanted cell proliferation comprising:

providing a differentiation agent to a cell of a subject to produce a differentiated cell;

providing the cell with a light emitting agent;

activating said agent; and

detecting an increase in light emission[a difference] between the differentiated cell and a control cell, thereby detecting the presence of a disorder characterized by unwanted cell proliferation.